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Contribution of roe deer to the epidemiology of anaplasmosis – new methodology and preliminary results

Key words: *Anaplasma phagocytophilum, Capreolus capreolus, Ixodes ricinus*, ticks and tick-borne diseases, epidemiology, EDEN Project, Germany

Introduction

Ticks (*Ixodes ricinus*) are obligatory blood-sucking ectoparasites which feed on any suitable vertebrate host, from small mammals and birds to large mammals, including humans. As a consequence of this absence of host specificity, ticks are vectors of many pathogens. Some of these pathogens are a cause of concern to both human and veterinary medicine. This is the case of anaplasmosis, which has been recorded in humans (Chen et al., 1994; Naranjo et al., 2006), dogs (Johansson et al., 1995), cattle (Joncour et al., 2005; Matsumoto et al., 2006; Naranjo et al., 2006; Woldehiwet, 2006), sheep, goat (Woldehiwet, 2006), horse (Bermann et al., 2002), donkey (Naranjo et al., 2006), moose (Jenkins et al., 2001), red deer (Naranjo et al., 2006), roe deer (Alberdi et al., 2000; Liz et al., 2002; Polin et al., 2004), and many species of birds (Naranjo et al., 2006). The causative agent of anaplamosis (also known as granulocytic ehrlichiosis) is *Anaplasma phagocytophilum*. This bacterium, also known under various synonymic names, is an obligatory intracellular parasite. It is commonly found in the blood, inside leukocytes (fig. 1). Ticks are often carriers of *A. phagocytophilum*. In Bavaria, prevalence may reach values above 5 % in questing ticks (de Mendonça et al., 2008). Considering the fact that tick density may be very high in suitable habitats, the risk of being bitten by an infectious tick is not negligeable. Prevalence estimates of *Anaplasma* infection in questing ticks imply that most ticks become infected through their nymphal blood meal. Indeed, prevalence is systematically and significantly much higher in adults than in nymphs (de Mendonça et al., 2008). This very strongly suggests that circling between immature and adult ticks oc-

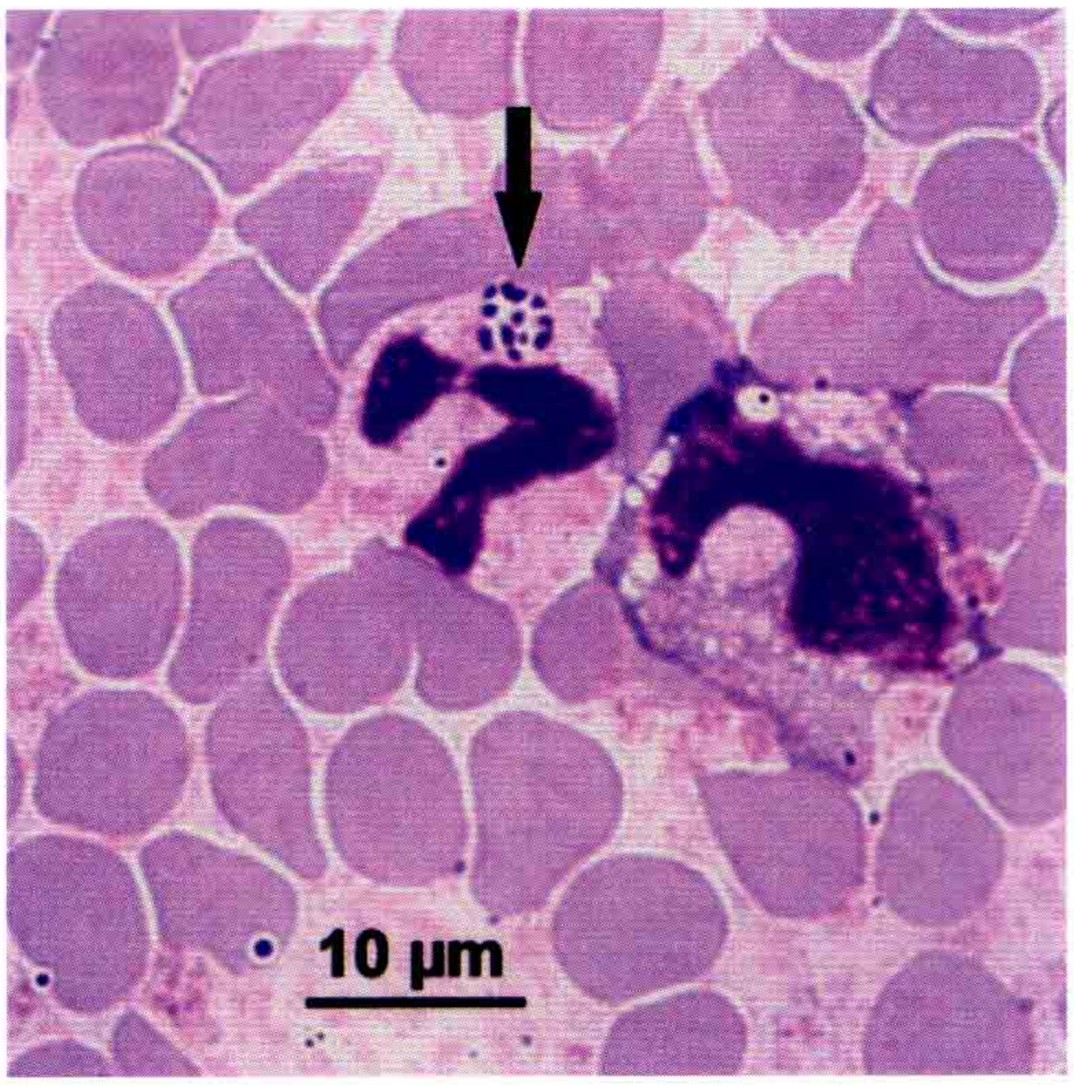

Fig. 1 Anaplasma phagocytophilum (arrow) is an obligatory intracellular bacterium commonly found inside leukocytes.

curs through cofeeding. The main amplification host must therefore simultaneously harbour larvae, nymphs and adult ticks. Small mammals, song birds, and deer are known sylvatic hosts to ticks. However, rodents are host to very few nymphs and hardly any adult tick (de MENDONÇA, 2003, 2005). Furthermore, the prevalence of *A. phagocytophilum* is extremely low in European rodents (P.G. de MENDONÇA, unpublished). Therefore, rodents are no major reservoir for this pathogen in Europe.

Cofeeding is common on thrushes and blackbirds, however, it usually involves larvae and nymphs (P.G. de MENDONÇA, unpublished). As deer (*Capreolus capreolus*, fig. 2) is known to host simultaneously all three tick stages in Bavaria, and as prevalence of *A. phagocytophilum* in deer in Germany is very high (above 45 %, based on PCR diagnosis) and varies with geographical location (P.G. de MENDONÇA, unpublished), we aimed at quantifying the potential for cofeeding on deer.

Fig. 2 Roe deer (Capreolus capreolus) is a reservoir host for Anaplasma phagocytophilum.

Materials & Methods

Activity of questing ticks:

Tick activity is routinely monitored in Bavaria and Thuringia over several 100 m long transect lines. This is done by dragging a white 1 m^2 flannel flag over soil and vegetation on a monthly basis. Every 2.5 m the flag is inspected and ticks collected and preserved for taxonomic and molecular investigations.

Quantitative sampling of ticks parasitizing deer:

Where feasible, the entire deer pelt is collected and spread out inside a white plastic tray. This first tray is then placed in the middle of a second white tray filled with water (fig. 3). Ticks crawling away from the pelt eventually fall into the water where they are easily detected and collected. Double-sided sticky tape on the rim of the external tray prevents any tick from escaping. Ticks are thus quantitatively collected.

Estimating deer abundance:

Estimates of deer abundance are obtained by the distance transect method (CAUGHLEY, 1977).

Fig. 3 The nested white tray technique makes it possible to quantitatively sample ticks from deer pelt.

Results

More than 150 deer samples were collected in Bavaria and Thuringia since June 2007. We present here only a subset of the analyses of these data. Most deer (90 %) were host to ticks. Tick-free deer were found in winter months only. Infested deer were usually hosts to both immature and adult ticks. All infested deer were host to adult ticks, whereas 90 % of them also hosted nymphs, and more than 70 % of them also hosted larvae. Nymphs were numerically

dominant (up to 210 nymphs per deer), followed by larvae (up to 199 larvae per deer), whereas adults were the least abundant, although the most prevalent instar (up to 157 adult ticks per deer). Most interestingly, all tick stages actually fed on deer. Fully engorged larvae, nymphs and adult ticks were indeed collected from deer. Furthermore, the larvae-to-nymphs, larvae-to-adults, and nymphs-to-adults ratios reach fairly high values (up to 48, 13, and 25 respectively). This demonstrates the high potential for cofeeding on deer. The intensity of tick infestation on deer is a function of questing activity and deer abundance. Tick activity has a positive impact on infestation intensity, whereas deer abundance has a diluting effect (fig. 4). No simple rectilinear model describes these relations satisfactorily, whereas a curvilinear model explains more than 40 % of the observed variability.

Discussion

The nested white tray technique proved useful to quantitatively sample ticks from deer pelt. This method made it possible to demonstrate that deer is host to numerous larvae, nymphs and adult ticks, and that all three stages actually feed on deer blood. We thus demonstrated that the potential for immature ticks to become infected with *Anaplasma phagocytophilum* while cofeeding on deer is very high. Deer is a common species reaching locally high densities. Furthermore, prevalence of anaplasmosis in deer is very high. Deer is therefore a source of food for ticks, thus favouring their reproduction, as well as a source of infection. In other words, deer is a maintenance host for ticks, and an amplification host for *Anaplasma phagocytophilum*.

Reducing deer density through culling may reduce absolute tick density, however, it increases cofeeding potential, as fewer deer are available to questing ticks. Culling may therefore turn out to be counterproductive, unless deer is fully eradicated. Such an extreme action is however not desirable. Foresters and hunters are routinely exposed to potentially infectious tick bites. However, hunters are exposed to an additional risk of infection while eviscerating deer carcasses. Indeed, the contact between damaged hand skin and infected deer blood was suggested as an additional route of infection for hunters (Bakken et al., 1996). Tick repellent and gloves should therefore be used by hunters handling deer carcasses.

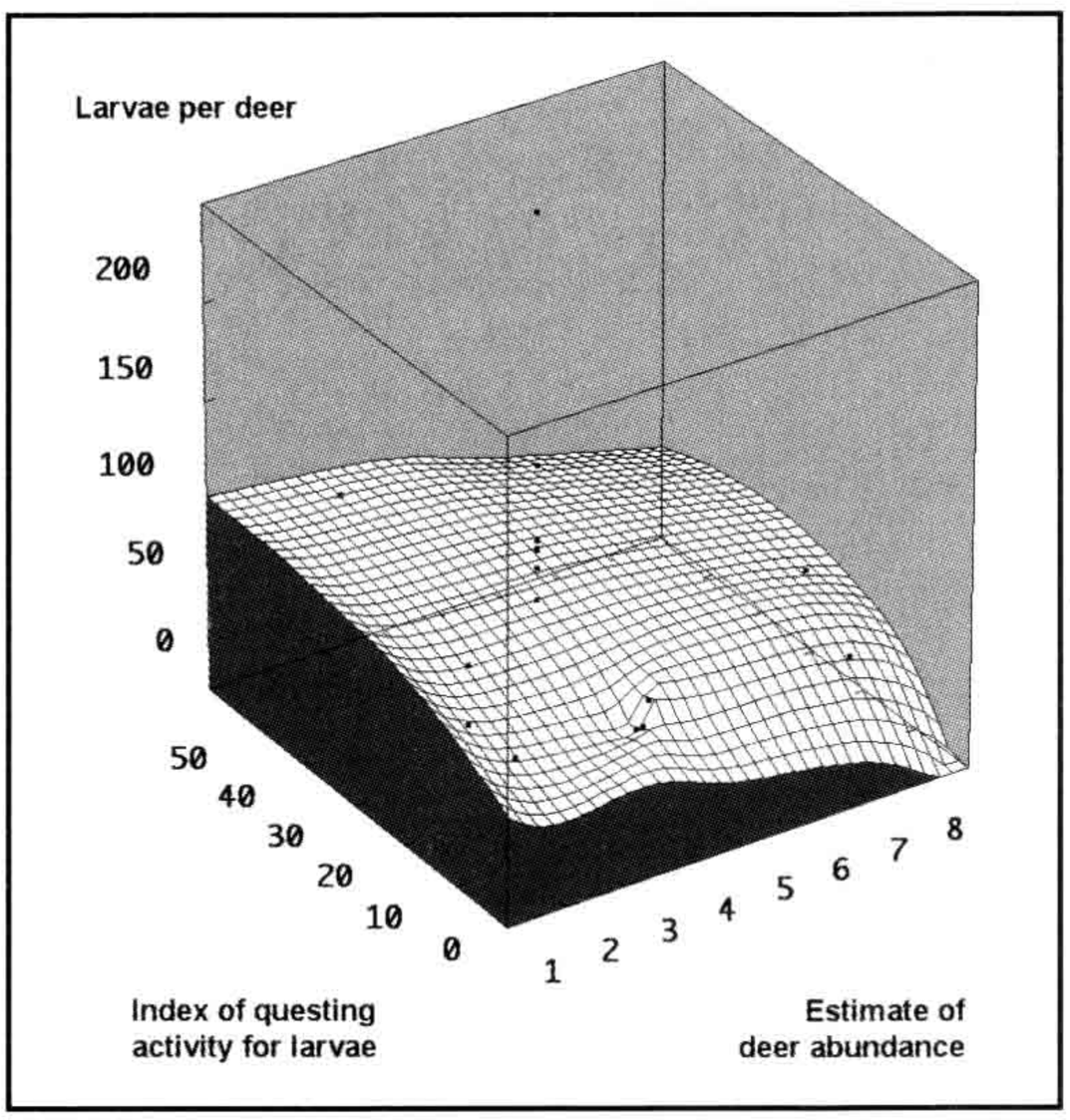

Fig. 4 Impact of deer abundance (estimated number of individuals per 1 km²) and index of questing activity for larvae (number of larvae collected on a 100 m² transect) on larval infestation on deer (based on the post mortem examination of 20 deer, using the nested white tray technique).

Individual contributions

Sampling of questing ticks: JH & PGM; Deer sampling: JH & PGM; Tick extraction from deer pelt: JH & PGM; Distance sampling (estimation of deer abundance): JH; Computer modelling: PGM.

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Summary

We demonstrated that the potential for immature ticks to become infected with *Anaplasma phagocytophilum* while cofeeding on roe deer is very high. Indeed, prevalence of *A. phagocytophilum* is very high in deer, and all three tick stages commonly feed on deer. Deer is a maintenance host for ticks, and an amplification host for *A. phagocytophilum*. Reducing deer density through culling may reduce absolute tick density, however, it increases cofeeding potential.

Zusammenfassung

Die Rolle des Rehwildes in der Epidemiologie der Anaplasmose – neue Methodologie und erste Ergebnisse

Wir haben aufgezeigt, dass das Potential, dass sich immature Zecken auf einem Reh durch Cofeeding mit *Anaplasma phagocytophilum* infizieren, sehr hoch ist. Tatsächlich ist die Prävalenz für *A. phagocytophilum* beim Reh sehr hoch und alle drei Zeckenstadien saugen im Allgemeinem auf Rehen. Rehe sind ein Hauptwirt für Zecken und ein „Verstärkerwirt" für *A. phagocytophilum*. Durch Abschuss die Rehdichte zu verringern, kann womöglich die absolute Zeckendichte reduzieren, aber es erhöht das Risiko von Cofeeding.

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